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Article

Zinc–cadmium interaction affects growth, metal accumulation, photosynthesis, and antioxidant activity in two mung bean varieties

Md Harunur Rashid[1,2*], Mohammad Mahmudur Rahman[1,2], Md Imran Ullah Sarkar[1,2,3] and Ravi Naidu[1,2]

[1]Global Centre for Environmental Remediation, The University of Newcastle, Callaghan, NSW 2308, Australia
[2]crc for Contamination Assessment and Remediation of the Environment (crcCARE), ATC Building, The University of Newcastle, Callaghan, NSW 2308, Australia
[3]Bangladesh Rice Research Institute, Gazipur, Bangladesh

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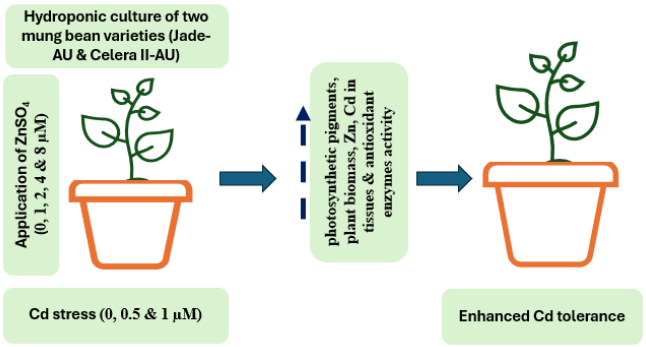
Hydroponic culture of two mung bean varieties (Jade-AU & Celera II-AU)
Application of ZnSO4 (0, 1, 2, 4 & 8 µM)
Cd stress (0, 0.5 & 1 µM)
photosynthetic pigments, plant biomass, Zn, Cd in tissues & antioxidant enzymes activity
Enhanced Cd tolerance

Highlights

- *Limited data on Zn and Cd distribution in mung beans under Zn supply and Cd stress.*
- *Zn–Cd interaction increased Cd uptake and Zn biofortification in mung beans.*
- *Zn reduced root Cd at low Cd levels but failed to limit shoot accumulation.*
- *Zn improved mung bean growth by boosting antioxidative enzymes under Cd stress.*

Abstract

Zinc (Zn) is an essential element, and zinc sulfate ($ZnSO_4$) has been extensively applied to crops to combat its deficiency. Moreover, adding Zn brings benefits by reducing cadmium (Cd) accumulation by crops, although its impact on Cd uptake is inconsistent in the literature. Two promising mung bean (*Vigna radiata*) varieties, released as Jade-AU and Celera II-AU by the Australian Mung Bean Association, were grown hydroponically to examine the effects of $ZnSO_4$ application on Cd accumulation in tissues. This was done by measuring photosynthetic pigments, plant biomass production, Zn and Cd accumulation, and antioxidative enzyme systems. Seven-day-old seedlings were exposed to different levels of Zn (0, 1, 2, 4, and 8 µM) and Cd (0, 0.5, and 1 µM) for 14 days. While the addition of Zn significantly ($p < 0.01$) enhanced photosynthetic pigments, plant biomass, and Zn and Cd concentrations in tissues, the prevalence of Cd revealed damaging outcomes. Zn accumulation in shoots increased as much as 6-fold (95 mg kg^{-1} in Jade-AU) with the application of Zn alone, while adding Cd restricted the trend. In contrast, Zn accumulation in roots rose 28-fold (146 mg kg^{-1} in Celera II-AU) when both Zn and Cd were present. Cd concentration in the shoot rose by as much as 22-fold (1.8 mg kg^{-1} in Celera II-AU) with the application of Cd alone and virtually tripled (5.42 mg kg^{-1} and 6.99 mg kg^{-1}, in Jade-AU and Celera II-AU, respectively), when Zn and Cd interacted synergistically. Zn addition restricted Cd accumulation in roots under low-Cd level (0.5 µM), while it was unable to limit the translocation of Cd to shoots. Zn application significantly improved antioxidant enzyme activities, with increases

*Corresponding author: harunbari05@gmail.com (MHR)
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observed up to 13% for APX, 41% for CAT, 40% for POD, and 35% for SOD relative to the control. This refers to both varieties of mung beans, indicating the benefit of Zn application in nullifying oxidative stress and maintaining plant growth. Of the two varieties, Jade-AU displayed less Cd translocation from the roots to shoots and produced higher biomass, demonstrating its Cd-tolerant advantages.

1 Introduction

Cadmium (Cd), a trace element and non-essential metal, is recognized as a Group A human carcinogen and exhibits phytotoxicity (Hamid et al., 2019). It prevails naturally in soils at trace levels and also presents as an impurity in phosphate fertilizers (Sheppard et al., 2009), refining of Zn ores (Elinder, 1985), and agricultural and mining operations (Ali et al., 2014; Murtaza et al., 2015). Cd is absorbed by plants because it is pervasive in the environment, although it is not essential for plants' function. Compared to other toxic elements, Cd readily translocate to the edible portion of plants to levels harmful for human consumption without showing any adverse effect on plant growth. Thus, it easily enters the food chain via crops (Clemens et al., 2013; Martos et al., 2016) and crop-based products through diet (Rietra et al., 2017; Nordberg et al., 2018), threatening human and animal health (ANZFA, 1998; Jackson and Alloway, 2017).

Besides individuals with significant exposure to tobacco use or occupational environments, diet is the primary source of Cd exposure (EFSA, 2009; Clemens et al., 2013). Chronic exposure to Cd from foods above acceptable levels (0.2 mg kg^{-1}) may lead to human health risks, for instance, damages to the skeletal, renal, and reproductive systems and causes kidney, lung, and other types of cancers (IARC, 2012; IARC, 2018). Excess Cd accumulation in plants causes toxicity at both physiological and molecular levels (Bayçu et al., 2017; Qayyum et al., 2017), induces oxidative stress, and suppresses antioxidant compounds. It manifests toxicity symptoms like chlorosis and leaf rolling (Gallego et al., 2012), while also reduces root and shoot growth, dry matter production, and photosynthetic pigment levels (Rizwan et al., 2016; Younis et al., 2016). Additionally, it alters stomatal conductance and impairs various enzyme activities in plants (Sarwar et al., 2014; Abbas et al., 2017).

Zinc is responsible for the synthesis of hundreds of enzymes related to plant growth and proper physiological functioning in human organ systems (Zhao et al., 2014; Parashuramulu et al., 2015). It also plays a vital role in the physiological and biochemical processes of plants (Qiao et al., 2015). Moreover, Zn plays a crucial role in regulating and maintaining gene expression essential for plant tolerance to environmental stresses (Cakmak, 2000). Zn deficiency accelerates Cd adsorption in the human body (Chaney, 1999) as well as its absorption by plants (Oliver et al., 1994). Release of phytosiderophores from roots of Zn-deficient plants occurs, and this can in turn trigger enhanced root uptake and translocation of Cd (Oliver et al., 1997; Köleli et al., 2004).

Cadmium and Zn are chemically analogous and Zn influences Cd uptake in plants by competing for available binding sites in soil and root surfaces (Christensen, 1984; Zare et al., 2018). This demonstrates both antagonistic (Tammam et al., 2016; Murtaza et al., 2017) and synergistic effects (Cojocaru et al., 2016) on each other during plant uptake. Their relationship varies based on the doses of Cd and Zn, exposure duration (Köleli et al., 2004), plant species (Fahad et al., 2015), plant organs/tissues (Sarwar et al., 2014; Tkalec et al., 2014), plant age (Lefèvre et al., 2009), source (Zhang and Song, 2006), and application method of Zn fertilizer (Forster et al., 2018), along with the availability of other elements in the soil (Liu et al., 2007).

Sarwar et al. (2014) discovered both soil and foliar Zn applications (15 and 30 mg Zn kg^{-1} as soil, 0.3% and 0.6% Zn as foliar) effectively reduced Cd in wheat grain grown in Cd-enriched soil (30 mg kg^{-1}). Specifically, a lower foliar dose (0.3%) was more prominent despite an approximately 25% increase in shoot Cd (25 mg kg^{-1}) being observed. Interactions between Zn and Cd accelerate Cd concentration in adult tobacco plants, although the effect was non-significant for seedlings (Tkalec et al., 2014). Different findings were observed by Köleli et al. (2004) in bread and durum wheat, where the addition of 10 and 25 mg kg^{-1} of Zn to soil enriched with 10 mg $kg^{-1}$of Cd led to elevated Cd levels in the shoots at 35 days after sowing, whereas a reduction was observed at 65 days after sowing. Zn addition at a low level (10 µmol L^{-1}) protects plants from Cd stress by diminishing the Cd uptake, chlorophyll breakdown, lipid peroxidation and enhancing antioxidant enzymes activities (POD, SOD, CAT, APX, GR), whereas a higher level of Zn (>50 µmol L^{-1}) accelerates oxidative stress in combination with Cd (Cherif et al., 2011). Zn could reduce Cd uptake in plants in many ways by affecting precipitation and adsorption (Zaccheo et al., 2006), competing with Cd for the same membrane transporters (Zhong-qiu et al., 2005; Tlustos et al., 2006), and facilitating Cd sequestration in vegetative plant parts. This helps to avoid its translocation to edible parts by increasing plant biomass and alleviating physiological stress (Jalloh et al., 2009). As an essential element for plants, Zn can positively influence plant growth under Cd stress compared to plants grown without Zn application (Garg and Kaur, 2013; Brennan and Bolland, 2014b).

Most of the studies on the interactions between Zn and Cd are based on cereal crops (Huang et al., 2019; Farooq et al., 2020), but virtually nothing has been published on pulse crops, despite the fact that they are just as important as cereals. The information about the antioxidant enzyme activities produced by Zn supply and the presence of Cd contamination in mung beans is also scarce. This gap may

partly stem from the assumption that findings from cereals can be generalized, although pulses likely differ in their root architecture, membrane transport systems, and metal-binding capacities, which could influence uptake and translocation of Zn and Cd. They also serve as a rich source of micronutrients such as Fe, Zn, and Ca. Mung bean is one of the world's most vital pulse crops, but it is very sensitive to Cd stress. In the present study, we compared two mung bean varieties at the vegetative stage to investigate the influence of Zn-Cd interactions on plant growth, photosynthetic properties, accumulation patterns of Zn and Cd, and antioxidant enzyme activities under Cd stress and increasing Zn applications.

2 Materials and methods

2.1 Plant growth conditions

Two mung bean cultivars, Jade-AU and Celera II-AU, were obtained from the Australian Mung Bean Association. Jade-AU is a tall variety (100-seed weight: 6–7.3 g) with weather and lodging resistance but moderately susceptible to powdery mildew, tan spot, and halo blight (AMA, 2015). Celera II-AU is a medium-height variety (100-seed weight: 3.2–3.5 g) with similar weather and lodging resistance, but it is also prone to halo blight, tan spot, and powdery mildew. Seeds were sterilized with 3% (v/v) hydrogen peroxide, washed with Milli-Q water, and placed in 1L pails with perforated lids. Seeds were placed into holes wrapped with cotton with their bottoms suspended in Milli-Q water to facilitate germination.

After 7 days, half-strength Hoagland solution (pH 5.6) was added to the pails to support seedling acclimatization, with the solution replaced every two days. After acclimatization, Zn (0 for control, 1, 2, 4, and 8 µM as $ZnSO_4.7H_2O$) and Cd (0 for control, 0.5, and 1 µM as $Cd(NO_3)_2{\cdot}4H_2O$ were added. The plants were maintained for 14 days in the nutrient solution containing both Zn and Cd. Zn and Cd treatments were chosen for analysis based on their moderate level of growth inhibition. The experiment was conducted in a greenhouse under a completely randomized design with four replications, maintaining environmental conditions of 25/20°C (day/night), 75% relative humidity, and a 16/8 h light/dark photoperiod.

2.2 Plant sampling and analysis

After 21 days, plants were harvested, and roots and shoots were separated using a stainless-steel cutter. To remove root surface Cd^{2+}, roots were soaked in 20 mM Na_2-EDTA for 20 minutes, then rinsed with Milli-Q water. Some samples were flash-frozen (for 5 minutes) in liquid nitrogen and stored at −80°C for subsequent measurements, while others were oven-dried at 72°C for 48 hours to record dry biomass and analyze Zn and Cd concentrations. Dried samples were ground, and 0.25 g was digested in a Teflon vessel with 3 mL nitric acid and 2 mL hydrogen peroxide using a microwave digester (CEM, MARS 6, USA). After overnight pre-digestion, solutions were diluted to 10 mL, filtered (PTFE 0.45 µm), and analyzed via inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, Japan). Quality control included blanks, duplicates, calibration verification, and standard reference materials.

2.3 Determination of chlorophyll

Chlorophyll concentration in mung bean leaves was determined using the dimethyl sulfoxide (DMSO) method (Manolopoulou et al., 2016). Leaf tissue (0.1 g) was incubated in 10 ml DMSO at 60–65°C for 1 hour until fully decolourized, then cooled and filtered. Absorption was measured using UV–vis spectrophotometer (Thermo Scientific BioMate 3S) at 665 nm and 648 nm, and blank determination was performed using DMSO. The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were expressed as fresh weight (mg g^{-1}, fw.) as per the following formulae (Barnes et al., 1992):

$$\text{Chlorophyll a (mg g}^{-1}\text{ fw.)} = 14.85\ A_{665} - 5.14\ A_{64} \quad (1)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ fw.)} = 25.48\ A_{665} - 7.36\ A_{648} \quad (2)$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ fw.)} = 7.49\ A_{665} + 20.34\ A_{648} \quad (3)$$

where, A_{665} = absorption value at 665 nm, and A_{648} = absorption value at 648 nm.

2.4 Antioxidant enzyme assay

Enzyme extracts were prepared as described by Kamal and Komatsu, (2015). Fresh leaf tissue (250 mg) was homogenized in 1 mL of extraction buffer containing 25 mM potassium phosphate buffer (PPB, pH 7.8), 0.4 mM EDTA, 1 mM ascorbic acid, and 2% polyvinylpyrrolidone-40. The homogenate was centrifuged twice at 15,000 g for 20 min at 4°C. The resulting supernatant was used for enzymatic activity assays, with activity calculated according to Borker et al. (2020):

$$\text{Enzyme activity (Units L}^{-1}) = (\Delta\text{Abs} \times \text{Total assay volume}) / (\Delta t \times \varepsilon \times l \times \text{Enzyme sample volume}).$$

Where, Δt represents the incubation time (min), ΔAbs is the change in absorbance, ε is the extinction coefficient of the substrates (M^{-1} cm^{-1}), and l is the cuvette diameter (1 cm). Enzyme activity (Unit) is defined as the amount of enzyme that oxidizes 1 µmol of substrate per minute. The unit was then converted to U g^{-1} fw by calculation.

Ascorbate peroxidase (APX) activity was measured in a 1 mL reaction containing 25 mM PPB (pH 7.0), 0.25 mM ascorbic acid, 0.1 mM EDTA, 10 mM H_2O_2, and 0.1 mL enzyme extract. The decrease in ascorbic acid at 290 nm was monitored, with activity calculated using an extinction coefficient (EC_{290}) of 2.8 mM^{-1} cm^{-1}.

Catalase (CAT) activity was measured in a mixture of 50 mM PPB (pH 7.0), 15 mM H_2O_2, and enzyme extract.

H_2O_2 decomposition was detected at 240 nm after 10 min using a DU 730 UV–vis spectrophotometer (Beckman Coulter, CA, USA), applying an EC_{240} of H_2O_2 (40 mM^{-1} cm^{-1}).

Peroxidase (POD) activity was measured in a reaction containing 100 mM PPB (pH 7.0), 2% H_2O_2, 4% p-phenylenediamine, 5N H_2SO_4, and enzyme extract. The reaction was initiated by adding the enzyme and stopped after 10 min with 5N H_2SO_4. Blanks contained H_2SO_4 before enzyme addition. Samples were stored at 4°C for 30 min, and absorbance at 485 nm was recorded, with activity calculated using EC_{485} of p-phenylenediamine (2.8 mM^{-1} cm^{-1}).

Superoxide Dismutase (SOD) activity was determined in a reaction with 200 mM PPB (pH 7.8), 250 µM nitro blue tetrazolium (NBT), 10 µM riboflavin, 0.1% tetramethylethylenediamine, and enzyme extract. Activity was based on the inhibition of NBT photoreduction at 560 nm using a UV–vis spectrophotometer (EC_{560} = 2.1 mM^{-1} cm^{-1}). One unit of SOD was defined as the quantity of enzyme required to achieve 50% inhibition of the reaction rate under the assay conditions.

2.5 Standard reference material analysis

To validate the results, certified spinach leaf material (NIST 1573a) was used as a standard reference. It underwent the same digestion process as the plant samples, yielding recovery rates of approximately 91% for Zn and 92% for Cd.

2.6 Statistical analyses

Statistical analysis was performed using JMP Pro 14.2.0 (JMP, 2019), using a three-way ANOVA with Student's t-test for comparisons. Figures were created in GraphPad Prism 9.0.0. Standard errors (n = 4) were calculated using Microsoft Excel.

3 Results

3.1 Photosynthetic pigments

The two varieties displayed significant differences in chlorophyll a ($p < 0.01$), chlorophyll b ($p < 0.01$), and total chlorophyll ($p < 0.05$) concentrations (**Table 1**). Of the two varieties, Jade-AU accumulated more chlorophyll a than Celera II-AU (**Fig. 1a**). The main effect of Cd significantly ($p<0.01$) decreased chlorophyll a concentration in both varieties (Table 1). However, the breakdown of chlorophyll a due to Cd stress was more prominent in Celera II-AU than that of Jade-AU. In Celera II-AU, the addition of Cd at

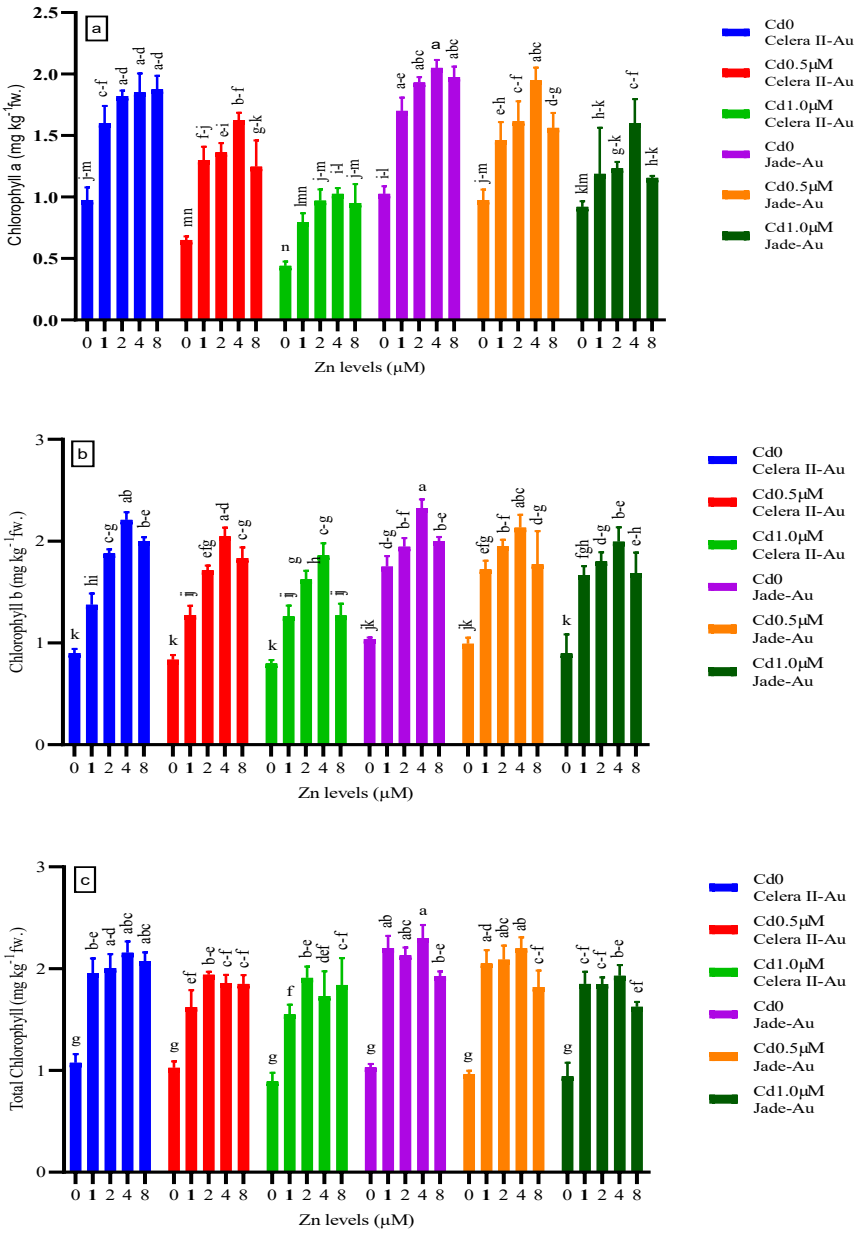

Figure 1. *Effect of Zn and Cd on photosynthetic pigments (mg g-1, fw): (a) chlorophyll a, (b) chlorophyll b, and (c) total chlorophyll contents of mung beans.*

Table 1. *Probability values (p-values) for key parameters in mung bean affected by varieties, varying Zn and Cd treatments and their interactions.*

Parameters	Variety	Zn	Cd	Zn x Cd	Variety × Zn	Variety x Cd	Variety × Zn x Cd
Chlorophyll a	0.0001	0.0001	0.0001	0.101	0.770	0.057	0.975
Chlorophyll b	0.0001	0.0001	0.0001	0.273	0.118	0.556	0.765
Total chlorophyll	0.035	0.0001	0.0001	0.847	0.011	0.530	0.985
Root dry matter	0.003	0.0101	0.0001	0.92	0.294	0.0003	0.938
Shoot dry matter	0.0001	0.0001	0.0001	0.541	0.167	0.046	0.733
Zn in roots	0.002	0.0001	0.0001	0.0001	0.231	0.996	1.00
Zn in shoots	0.0001	0.0001	0.0001	0.0001	0.015	0.360	0.009
Cd in roots	0.0001	0.0001	0.0001	0.0001	0.105	0.0001	0.001
Cd in shoots	0.0001	0.0001	0.0001	0.0001	0.014	0.0002	0.489
APX Activity	0.0001	0.0001	0.0001	0.999	1.00	0.998	1.00
CAT Activity	0.0001	0.0001	0.0001	0.474	1.00	0.933	1.00
POD Activity	0.0001	0.0001	0.0001	1.00	1.00	0.992	1.00
SOD Activity	0.0001	0.0001	0.0001	0.989	1.00	0.999	1.00

0.5 µM and 1.0 µM reduced the chlorophyll a concentration by approximately 33% (0.650 mg g^{-1}, fw.) and 55% (0.441 mg g^{-1}, fw), respectively, compared with control (0.975 mg g^{-1}, fw.). Meanwhile, the reduction of chlorophyll a in the Jade-AU variety was only 5% and 10%, respectively. In contrast, under no Cd, increasing levels of Zn linearly and significantly ($p < 0.01$) enhanced chlorophyll a concentration in the Celera II-AU variety, while in Jade-AU, it began to decline after Zn levels of 4 µM. At the highest Zn level (8 µM), chlorophyll a concentration rose to 1.975 mg g^{-1}, fw. in Jade-AU and 1.875 mg g^{-1}, fw. in Celera II-AU, almost double the levels of their respective controls. Interaction between Zn and Cd was not a significant factor for the accumulation of chlorophyll a in mung bean leaves. However, Zn supply up to 4 µM continued to increase the amount of chlorophyll contents in both varieties when Cd was present at both lower and higher concentrations. Interaction of variety, Zn and Cd influenced insignificantly on the concentration of chlorophyll b in mung bean leaves. Accumulation of chlorophyll b was significantly ($p < 0.05$) higher for Jade-AU than Celera II-AU, irrespective of treatments applied (**Fig. 1b, Table 1**). The effect of Cd was responsible for shrinking the concentration of chlorophyll b, about 11% for Celera II-AU and 14% for Jade-AU. Up to 4 µM of Zn application, chlorophyll b content showed a continuous increase followed by a decline. This increase was approximately 145% higher (2.21 mg g^{-1}, fw.) in Celera II-AU and 124% higher (2.33 mg g^{-1}, fw.) in Jade-AU when compared to their respective controls (0.900 mg g^{-1}, fw. and 1.04 mg g^{-1}, fw.). The negative influence of Cd on chlorophyll b concentration was mitigated by adding Zn up to 4 µM. The effect of elevated Zn levels in preventing photosynthetic breakdown was more potent at a lower Cd level (0.5 µM) and weaker at a higher Cd level (1.0 µM).

Total chlorophyll content varied significantly due to the mean effect of variety ($p < 0.05$), Zn ($p < 0.01$), and Cd ($p < 0.01$) (**Table 1**). For the presence of Cd at 0.5 µM and 1.0 µM, concentration of total chlorophyll decreased by 5% and 17%, respectively, in the Celera II-AU and by 7% and 9%, respectively, in the Jade-AU when compared to the respective controls (**Fig. 1c**). In both varieties, the concentration of total chlorophyll was almost doubled ($p < 0.05$) than that of the controls due to increasing Zn level in the media. The interaction between Cd (0.5 µM) and Zn elevated total chlorophyll concentration by approximately 80% in Celera II-AU and 113% in Jade-AU as compared to the respective controls. However, the rate of increment was less evident at the higher Cd level (1.0 µM).

3.2 Root and shoot dry matter

The shoot dry matter yield was significantly influenced by the mean effect of Zn, Cd and variety ($p < 0.01$) (**Table 1**). Shoot dry matter production was 103% higher in Jade-AU than Celera II-AU at Zn and Cd control (**Fig. 2a**). The presence of Cd (1 µM) significantly reduced shoot dry matter weight by up to 35% for Jade-AU and 12% for Celera II-AU compared with the control. Contrasted to this, the added Zn significantly increased shoot dry matter weight, which reached a peak of 1.06 g at 4 µM Zn for Celera II-AU and 1.59 g at 8 µM Zn for Jade-AU. The interaction of Zn and Cd produced a larger yield of dry matter compared with the main effect of Cd and lower weight compared with the main effect of Zn.

Both varieties being investigated displayed significant ($p < 0.01$) variations in root dry matter production (**Fig. 2b**). Root dry matter was approximately 77% higher in

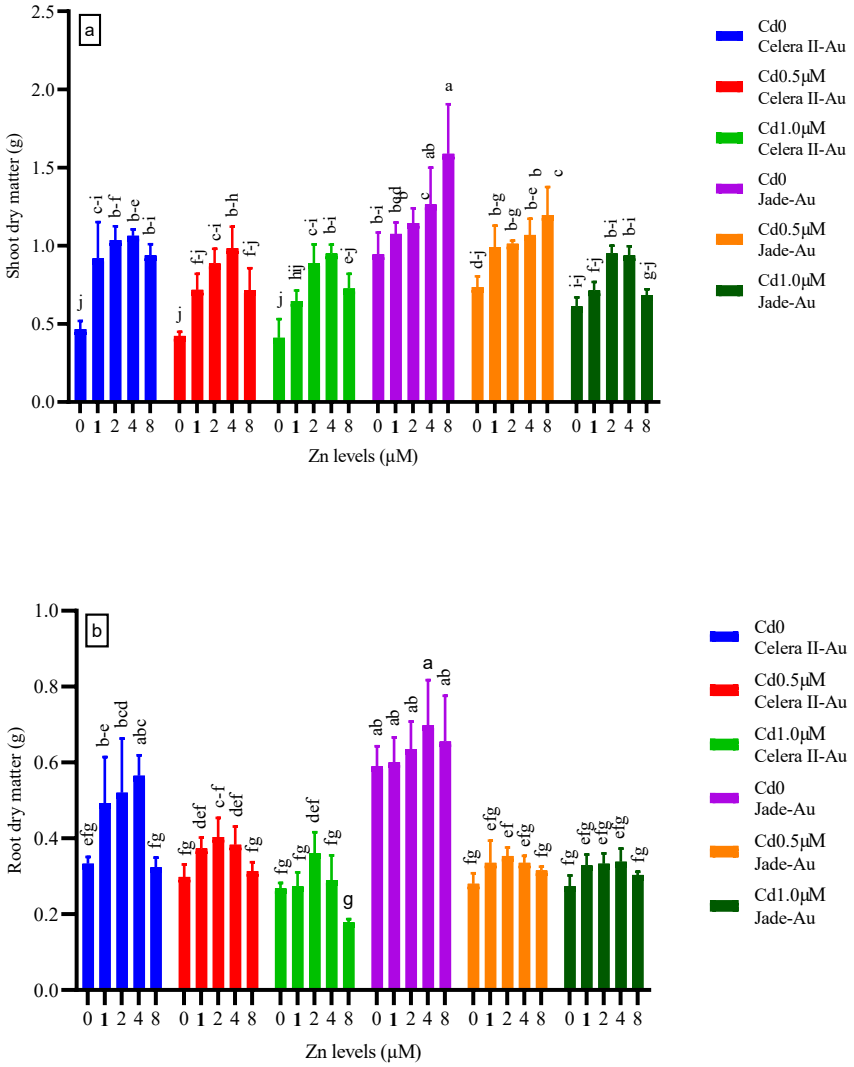

Figure 2. *Effect of Zn and Cd on (a) shoot and (b) root dry matter contents of mung beans.*

Jade-AU (0.590 g) compared with Celera II-AU (0.323 g) at baseline. However, root dry matter production varied with the addition of Zn and Cd both individually ($p < 0.01$) and not in combination (**Table 1**). Cd stress caused a significant ($p < 0.01$) decline in root dry matter production compared with control. The prevalence of Cd (0.5 µM and 1 µM) to the solution caused a reduction ($p < 0.01$) in root dry matter by 11% and 20%, respectively, in Celera II-AU compared with the control (0.333 g) and a decline of more than 50% in Jade-AU compared with the control (0.590 g). In both varieties, Zn (4 µM) application alone significantly enhanced ($p < 0.01$) root dry matter production, which reached a peak of 0.698 g in Jade-AU while it was 0.565 g in Celera II-AU. Although insignificant, the Zn supplementation mitigated the negative impact of Cd on root dry matter production. The highest root dry matter weight (0.698 g) was observed in Jade-AU with the addition of Zn only at 4 µM. Meanwhile, the smallest root dry matter weight (0.178 g) was observed in Celera II-AU with the combination of 8 µM Zn and 1 µM Cd.

3.3 Concentration of Zn in tissues

Significant differences ($p < 0.01$) appeared in shoot Zn concentrations between the two varieties (**Table 1**), where Jade-AU displayed a slightly higher accumulation (up to 10%) compared with Celera II-AU (**Fig. 3a**). The added Zn individually (1–8 µM) increased Zn accumulation significantly ($p < 0.01$) in shoots, more so in Jade-AU (108% at 1 µM to 616% at 8 µM) than in Celera II-AU (81% at 1 µM to 445% at 8 µM). Nonetheless, the presence of Cd to the solution held back the upward trend of shoot Zn in both varieties. While the mean effect of Zn (8 µM) increased shoot Zn concentration by 445% and 616% for Celera II-AU and Jade-

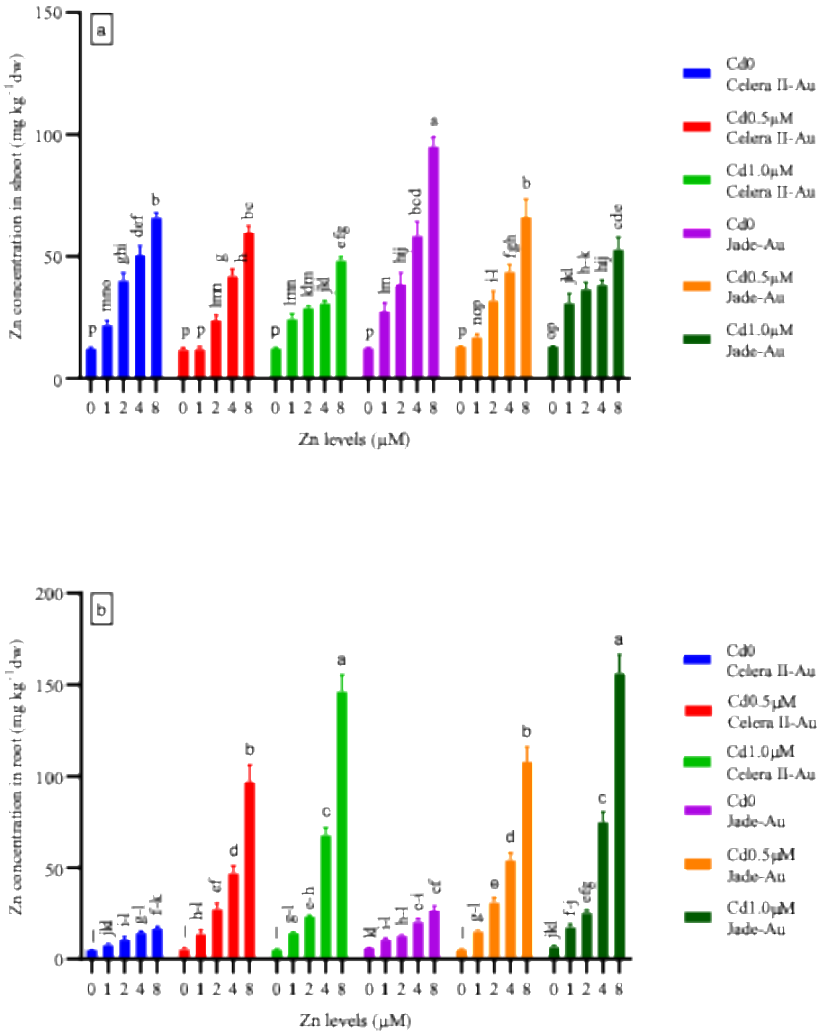

Figure 3. *Effect of Zn and Cd on Zn concentration in the (a) shoot and (b) root of mung beans.*

AU, respectively, its interaction with Cd (0.5 µM and 1.0 µM) halted these increases to 400% and 300% for Celera II-AU and Jade-AU, respectively.

The two varieties displayed a significant difference ($p < 0.01$) in the root concentration of Zn (**Fig. 3b**). In the control roots, Zn concentration was 6.08 mg kg^{-1} in Jade-AU and 5.03 mg kg^{-1} in Celera II-AU (approximately 21% lower). However, the presence of Zn and Cd did not lead to any significant variations in root Zn concentration. As expected, the added Zn (8 µM) significantly ($p < 0.01$) increased root Zn accumulation by 233% in Celera II-AU (16.75 mg kg^{-1}) and 340% in Jade-AU (26.75 mg kg^{-1}) compared with their respective controls (5.03 mg kg^{-1} and 6.08 mg kg^{-1}). The combination of Zn and Cd also linearly and significantly ($p < 0.01$) increased Zn concentration in roots (**Table 1**). At the highest interaction level (Zn 8 µM and Cd 1 µM), root Zn concentration reached the peak of 146 mg kg^{-1} in Celera II-AU and 156 mg kg^{-1} in Jade-AU. This was higher than root Zn concentration following only Zn application.

3.4 Concentration of Cd in tissues

Shoot Cd concentration was strongly influenced ($p < 0.01$) by mung bean varieties, applied Zn, and Cd (**Fig. 4a, Table 1**). In all treatments, including control, shoot Cd concentration was significantly lower (8–78%) in Jade-AU than in Celera II-AU. The sharp increase of Cd in shoots was presumably attributable to Cd being added to the solution. Under 0.5 µM and 1.0 µM Cd contamination, shoot Cd concentration increased by approximately 17-fold (1.39 mg kg^{-1}) and 22-fold (1.79 mg kg^{-1}), respectively, in Celera II-AU compared with the control (0.079 mg kg^{-1}) and 19-fold

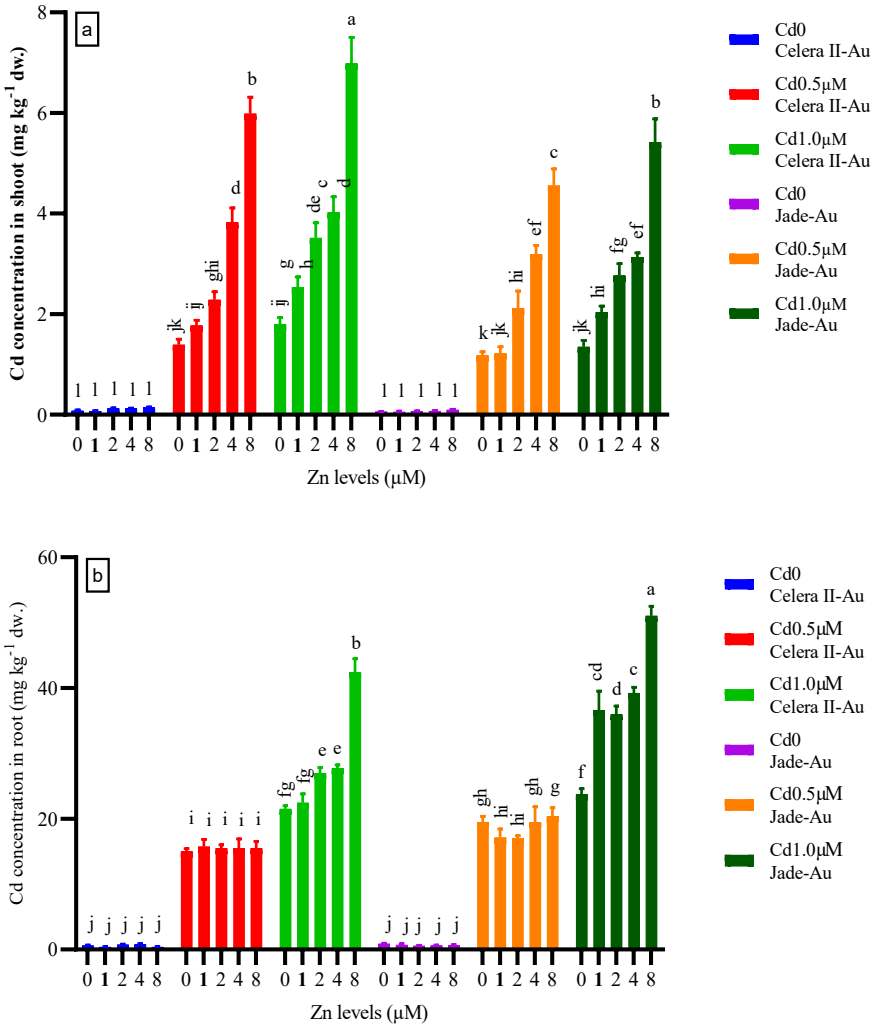

Figure 4. *Effect of Zn and Cd on Cd concentration in (a) shoot and (b) root of mung beans.*

(1.18 mg kg^{-1}) and 22-fold (1.35 mg kg^{-1}), respectively, in Jade-AU compared with the control (0.06 mg kg^{-1}). The combination of both Cd level and the increasing levels of Zn in solution sharply elevated ($p < 0.01$) the accumulation of Cd in shoots in both varieties (**Table 1**). Here, the highest Zn (8 µM) and Cd (1.0 µM) levels revealed the largest Cd accumulation (Celera II-AU: 6.98 mg kg^{-1}; Jade-AU: 5.42 mg kg^{-1}). The interaction effect of Zn and Cd was also more pronounced ($p < 0.01$) than the main effect of Cd.

Root Cd concentration varied significantly ($p < 0.01$) depending on the plant varieties, Zn, Cd, and all their interactions, except for the interaction between variety and Zn (**Table 1**). Of the two varieties, Jade-AU accumulated more Cd in the roots (up to 78%) for all treatments except for individual Zn at 2 µM and 4 µM (**Fig. 4b**). At control, Jade-AU demonstrated a 49% higher root Cd (0.893 mg kg^{-1}) compared with Celera II-AU (0.601 mg kg^{-1}). When the solution was spiked with 0.5 µM Cd, Cd accumulation in roots increased 24-fold (15.0 mg kg^{-1}) and 21-fold (19.5 mg kg^{-1}) for Celera II-AU and Jade-AU, respectively, compared with controls. Root Cd concentration rose significantly in the presence of higher Cd (1 µM) compared with less Cd (0.5 µM). At the lower Cd level (0.5 µM), increasing levels of

Zn restricted Cd accumulation in roots in both varieties. However, at the higher Cd level (1 µM), root Cd accumulation increased linearly ($p < 0.01$) with rising levels of Zn, reaching peaks of 51.1 mg kg^{-1} and 42.4 mg kg^{-1} in Jade-AU and Celera II-AU, respectively.

3.5 Effects of Cd and Zn on the activity of antioxidant enzymes

3.5.1 APX activity

APX activity was significantly ($p < 0.01$) affected by the mean effects of variety, applied Zn, and Cd contamination. (**Fig. 5a, Table 1**). APX activity geared up to 438.3 U g^{-1} fw. from 387.1 U g^{-1} fw. (control) and 400 U g^{-1} fw. from 353.2 U g^{-1} fw. (control) for Jade-AU and Celera II-AU, respectively, under Cd contamination (1 µM Cd). For both varieties, up to 4 µM of Zn supply enhanced APX activity (about 13%), relative to the control. However, about 26% higher APX activity was observed for the effect of interaction between Zn and Cd.

3.5.2 CAT activity

Under Cd stress and added Zn, the varieties of mung bean exerted a significant influence ($p < 0.01$) on CAT activity (**Table 1**). CAT activity gradually increased with a rising level of Zn, which was about 41% and 37% higher for Celera II-AU and Jade-AU, respectively, when compared to the control (**Fig. 5b**). Similarly, under Cd contamination conditions, it was evident that CAT activity soared. While CAT activity increased by about 7-8% under 0.5 µM Cd, its higher level (1 µM Cd) was responsible for the increase of 17-19%, as compared to the control. Interaction between Zn and Cd accelerated CAT activity tremendously, which was about 51% and 47% higher than respective controls for Celera II-AU and Jade-AU, respectively.

3.5.3 POD activity

Zinc and Cd treatments significantly ($p < 0.01$) increased POD activity in mung bean leaves in a concentration-dependent manner (**Table 1**). About 15% increased POD activity, i.e., 20.1 U g^{-1} fw. and 21.83 U g^{-1} fw. were observed in Celera II-AU and Jade-AU, respectively, due to added Cd (1 µM) compared to the control (**Fig. 5c**). With the increasing Zn being supplied to both varieties, POD activity was increased and reached its peak (24.53 U g^{-1} fw. for Celera II-AU and 26.1 U g^{-1} fw. for Jade-AU). A more profound impact was observed as a result of the interaction between Zn and Cd compared to the mean effect. When comparing to the control, about 50% increment was

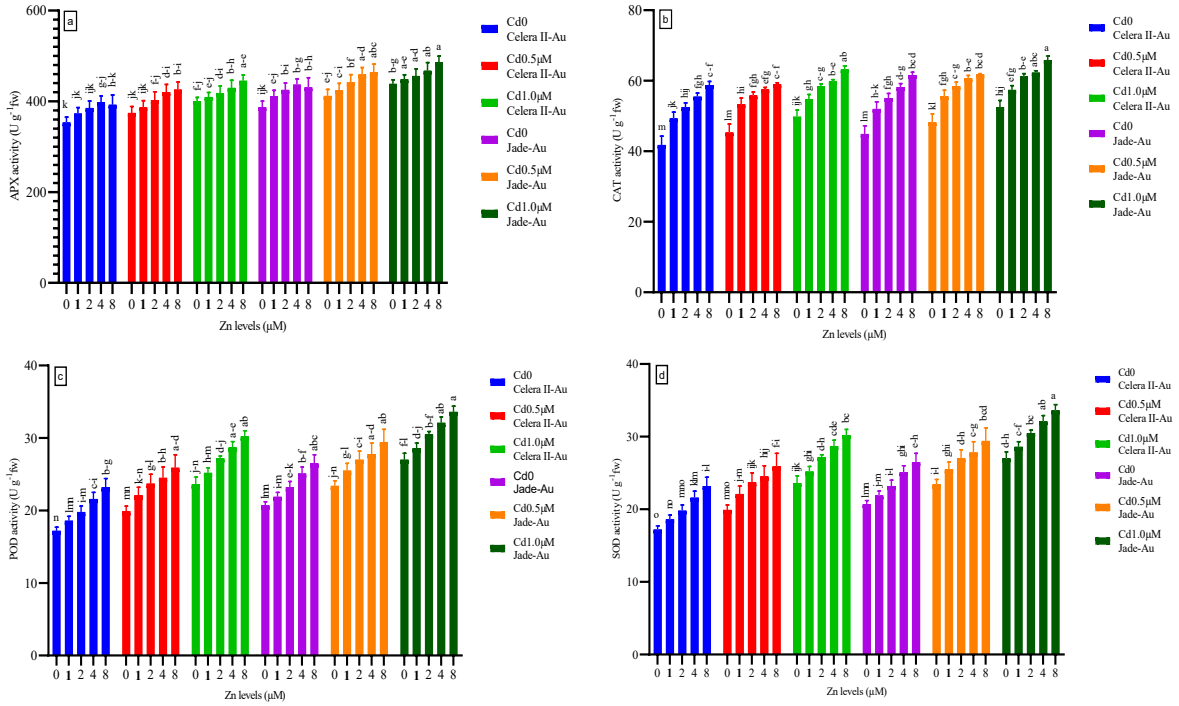

Figure 5. *Effect of Zn and Cd on antioxidant enzyme activities of mung beans: (a) APX, (b) CAT, (c) POD and (d) SOD.*

observed for the interaction of applied Zn (8 µM) and Cd (1 µM) concentrations.

3.5.4 SOD activity

Like other enzymes, the induced SOD activity was noted ($p < 0.01$) for variety, Zn and Cd in mung beans in a concentration-dependent manner (**Fig. 5d, Table 1**). For both varieties (Celera II-AU and Jade-AU), the Cd levels of 0.5 µM was responsible for about 13-16% higher activity of SOD amounting to 19.9 U g^{-1} and 23.4 U g^{-1}, respectively, in contrast to the control (**Fig. 5d**). Further Cd contamination almost doubled the SOD activity in both mung bean varieties. Furthermore, the added Zn gradually enhanced the activity of SOD from 17.2 U g^{-1} fw. (control) to 23.2 U g^{-1} fw. for Celera II-AU and from 20.7 U g^{-1} fw. (control) to 26.5 U g^{-1} fw. for Jade-AU. More than 60% increase in activities of SOD was obtained for mung beans because of the combined effect of Zn and Cd in solution.

4 Discussion

In this study, photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) increased when the Zn in solution rose to 4 µM (**Fig. 1a**). Samreen et al. (2017) observed similar results when investigating mung bean varieties grown in nutrient solution with and without Zn. Other studies showed that Zn deficiency disrupts chlorophyll synthesis, while Zn application increases the chlorophyll content in wheat (Safari et al., 2019). Zn serves as both a structural and catalytic component of proteins and enzymes, functioning as a co-factor in pigment biosynthesis (Balashouri and Prameeladevi, 1995). Zn was positively correlated with several parameters (e.g. net photosynthetic rate, protein and chlorophyll concentration, and nitrate reductase activity) in Japanese mint (Mentha arvensis) (Misra, 1992). In contrast, in a field study, Zn inhibited chlorophyll concentration in corn by interfering with iron metabolism (Rosen et al., 1977).

The application of a higher level of Zn (8 µM) resulted in a decline in chlorophyll pigments. This is supported by Manivasagaperumal et al. (2011), who investigated cluster beans grown in solutions with different Zn levels and observed a decline in both growth and photosynthetic pigments at higher Zn levels (> 25 mg L^{-1} $ZnSO_4$ solution). This may have been attributable to the fact that excess Zn prevents the incorporation of iron into protoporphyrin, the precursor of chlorophyll synthesis, resulting in a reduction in chlorophyll pigment (Granick, 1951). We observed an approximate 5–55% reduction in chlorophyll pigment (**Fig. 1a**) with Cd treatment, which agrees with other research findings (Muradoglu et al., 2015; Paunov et al., 2018; Safari et al., 2019). The reduction in chlorophyll content in plants caused by heavy metal stress has been linked to: i) the inhibition of key enzymes involved in chlorophyll biosynthesis (Qian et al., 2009), including δ-aminolevulinic acid dehydratase (Padmaja et al., 1989) and protochlorohyllide reductase (Van Assche and Clijsters, 1990); ii) the impairment of magnesium and iron supply (Küpper et al., 1996); and/or iii) the inhibited biosynthesis of chlorophyll and the generation of a type of senescence (Qian et al., 2009).

Jade-AU produced a significantly higher root and shoot biomass compared with Celera II-AU (**Fig. 2a, b**). Varietal differences in plants occur due to differences in their morphophysiological characteristics (Zorrig et al., 2013). These differences may have been attributable to the larger Zn concentrations in the tissues of Jade-AU compared with Celera II-AU. Moreover, Jade-AU may have a greater demand for Zn and a better response to Zn fertilization; thus, if adopted for cultivation, Jade-AU is expected to help solve the problem of human Zn deficiency.

The Jade-AU cultivar also accumulated more Cd in its roots and less Cd in its shoots compared with Celera II-AU. Therefore, Jade-AU was more tolerant to Cd stress (at 1 µM Cd) compared with Celera II-AU. This intra-species variability in Cd accumulation and tolerance has been previously observed in crops (Zorrig et al., 2013; Brennan and Bolland, 2014a). Cd tolerance in plants is associated with the activation of specific genes, such as *MAN3* and *AtFC1*, which regulate the synthesis of phytochelatins through glutathione-dependent pathways (Chen et al., 2015; Song et al., 2017). These phytochelatins bind Cd and facilitate its sequestration into vacuoles, contributing to Cd accumulation and enhanced tolerance. Additionally, Cd may also be sequestered in the apoplast, which can influence both its accumulation and the plant's tolerance response (Meyer et al., 2015). In this study, the application of Zn up to 4 µM increased root and shoot biomass, followed by a reduction under Cd stress conditions (0.5 µM and 1 µM) as well as Cd control (**Fig. 2a, b**). Cluster beans treated with low levels of Zn (10 mg kg^{-1} and 25 mg kg^{-1} as $ZnSO_4$) displayed higher germination, growth, and biomass yield than the control (Manivasagaperumal et al., 2011). The shoot biomass of wheat also increased with the application of Zn at 15 mg kg^{-1} but declined with a higher application of Zn (30 mg kg^{-1}) in Cd-contaminated soil (30 mg kg^{-1}). This strongly suggests that Zn at lower levels has remarkable stimulatory and nutritional features (Reichman, 2002). The positive effect of Zn supplementation on plant growth has been observed in many other plants (Dikkaya and Ergun, 2014). The drop in yield at higher Zn concentrations may be attributable to the toxic effect of Zn on plant growth (Atici et al., 2005).

In this study, regardless of the availability of Cd in solutions, high Zn levels have resulted in increased Zn concentrations in both roots and shoots (**Fig. 3**), supporting agronomic biofortification (Cakmak and Kutman, 2018). This finding aligns with existing literature, which indicates that agronomic biofortification with Zn promotes accumulation of Zn (Huang et al., 2019; Zhou et al., 2019; Farooq et al., 2020). Unlike roots, the presence of Cd in solution mitigated the increase in Zn concentration in shoots, which is similar to the findings reported for mature tobacco plants (Tkalec

et al., 2014). They confirmed that the interaction between Zn and Cd restricted Zn concentration in the shoots.

Interactions between elevated levels of Zn with both Cd levels consistently elevated Cd concentration in the roots and leaves of mung beans (**Fig. 4**). This may have been attributable to the high Cd and low Zn availability in the solution, leading to oxidative damage and exacerbating membrane permeability (Rady and Hemida, 2015), facilitating the non-selective uptake of Cd into root cells by mass flow. However, the effect of Zn application on tissue Cd concentration was not consistent and, in fact, depended on several factors. The addition of Zn to clay soil in one study conducted in Turkey increased the concentration of Cd in 45-day-old whole maize (Adiloglu et al., 2005). Cd concentration of the shoots of maize growing in loamy soil in China diminished with the application of Zn from three different sources [$ZnSO_4$, $Zn(NO_3)_2$ and $ZnCl_2$], while root Cd concentration increased with $ZnSO_4$ but decreased with $Zn(NO_3)_2$ and $ZnCl_2$ (Zhang and Song, 2006). The application of Zn to loamy, calcareous soil and foliage increased Cd concentration in wheat shoots but decreased Cd concentration in wheat grain. Meanwhile, the application of Zn spray to clay soil confirmed that a decline in Cd concentration in all tissues at different growth stages did occur, especially the booting stage (Saifullah et al., 2016).

Other studies have also discovered that Zn application reduces Cd concentration in wheat tissues (Hussain et al., 2019; Farooq et al., 2020). In their work, Farooq et al. (2020) observed that a high Zn content in wheat seeds was associated with a higher exclusion of Cd in wheat straw and grains. In contrast, although Zn application reduces Cd accumulation in wheat straw and grain, the biofortified variety accumulates more Cd than the standard variety (Hussain et al., 2019). Reduction of Cd by Zn application has also been observed in all rice tissues except roots (Huang et al., 2019). In tomato plants, interactions between Zn (10, 50, 100, and 150 µmol L^{-1} as $ZnCl_2$) and Cd (10 µmol L^{-1} as $CdCl_2$) gradually reduced leaf Cd concentration (Cherif et al., 2011). In the nutrient solution, the application of Zn at different levels showed a positive impact on Cd accumulation in roots and seedlings of tobacco plant (Tkalec et al., 2014).

Plants produce enzymatic as well as non-enzymatic antioxidants to safeguard cells against oxidative stress resulting from ROS production in oxidizing environment (Tripathy and Oelmüller, 2012). Both Zn and Cd enhanced the antioxidant enzyme activities of mung beans according to the results of this research (**Fig. 5**). In this study, the APX activities at control were 353.2 Ug^{-1} fw. and 387.1 Ug^{-1} fw. for Celera II-AU and Jade-AU, respectively (**Fig. 5a**), which were significantly boosted by the presence of Zn (about 13% over the control), Cd (about 13% over the control) and their interactions (about 26% over the control). About 250 to 400 U g^{-1} fw. APX activity was detected in a study conducted with mung beans (Leng et al., 2021), which is in line with our findings. Boosted APX activity was also observed in mung bean seedlings in another study (Hassan et al., 2021). CAT activity ranged from 41.8 Ug^{-1} fw. to 63.2 Ug^{-1} fw. and from 44.9 Ug^{-1} fw. to 65.9 Ug^{-1} fw. in Celera II-AU and Jade-AU, respectively, irrespective of Zn and/or Cd treatment (**Fig. 5b**). CAT activity in mung beans varied between 17.5 and 18.2 µM min^{-1} mg^{-1} protein (Farahani and Taghavi, 2016), which supports our study.

For both varieties of mung beans, POD and SOD activities were enhanced in our study. While we discovered POD activity from 17.48 Ug^{-1} fw. to 28.20 Ug^{-1} fw. in this study, about 30.4-32.6 µM min^{-1} mg^{-1} protein was reported in mung beans by Farahani and Taghavi (2016). In this study, SOD activity ranged from 17.2 Ug^{-1} fw. to 30.2 Ug^{-1} fw. and 20.7 Ug^{-1} fw. to 33.6 Ug^{-1} fw. in Celera II-AU and Jade-AU, respectively, where about 28.1–34.9% increased activities were evident due to added Zn. Higher SOD activity (138-139%) was found in mung beans for added Cd (Ramzani et al., 2017), whereas Cd increased about 37.2% of SOD activity in the present study. Regarding SOD activity in mung beans analyzed by Hassan et al. (2021), which supports our findings, while Leng et al. (2021) documented the opposite scenario. Added Zn enhanced antioxidant enzyme activities in different crops under Cd contamination, which justifies the necessity of applying Zn under Cd stress to boost enzyme activities, instead of no Zn application (Cherif et al., 2011; Rizwan et al., 2019). The activation of the antioxidant defense mechanism results from the expression of genes (Nair and Chung, 2014) and/or its relationship with various oxidative processes (Hossain et al., 2015). To sum up, the application of Zn fertilizer is of paramount importance for crops grown under Cd contamination to avert oxidative stress and ensure that production remains viable.

The observed synergistic interaction between Zn and Cd resulted in increased levels of Cd in various tissues of mung bean, particularly in the shoots, highlighting a complex interplay that likely involves both physiological and molecular mechanisms. One plausible physiological explanation is that Zn application, especially at sub-toxic or stress-alleviating doses, enhances plant vigor and root surface area, inadvertently promoting the uptake of other divalent cations, including Cd, via non-selective cation channels or shared transporters (Maser et al., 2001). Furthermore, Zn and Cd are known to compete for and sometimes co-utilize ZIP (ZRT/IRT-like Protein) and NRAMP (Natural Resistance-Associated Macrophage Protein) channels, which are known to mediate divalent metal uptake in various plant species (Milner et al., 2013). Zinc application might upregulate under Zn deficiency or Zn supplements (Lee and An, 2009). In rice, for example, OsIRT1 and OsZIP1 are upregulated under Zn deficiency and have been implicated in Cd uptake (Zheng et al., 2012). Although such transporters have not yet been characterized in mung beans, their existence and potential role in Zn–Cd interplay warrant further molecular investigation.

5 Conclusion

Compared with the Celera II-AU, Jade-AU displayed significantly higher values for chlorophyll a (up to 109%), chlorophyll b (up to 35%), root dry matter (up to 103%),

shoot dry matter (up to 74%), root Zn accumulation (up to 60%) and shoot Zn accumulation (up to 44%). Although Jade-AU accumulated more root Cd (up to 78%), significantly less accumulation was observed in shoots (up to 78%) compared with Celera II-AU, demonstrating the Cd-excluding capability of Jade-AU. Although the application of individual Zn significantly increased shoot Zn levels in both varieties (by 445% in Celera II-AU and 616% in Jade-AU), the effect was less prominent with the addition of Cd. However, the Zn–Cd interaction sharply enhanced root Zn accumulation in both varieties (up to 28 times for Celera II-AU and 25 times for Jade-AU) compared with the sole Zn application. Like root Zn, the accumulation of Cd in roots and shoots was far higher with the application of both Zn and Cd compared with Cd only, indicating a synergistic relationship between Zn and Cd. Despite the greater accumulation of Cd in tissues, shoot dry matter exhibited a significant upward trend. This might be due to the increased antioxidant enzyme activities for added Zn, which strengthens the hypothesis that Zn is essential in protecting plants from Cd stress by enhancing plant defense against Cd-induced oxidative stress. Further studies using other Zn sources and application methods should be conducted at field level, focusing on metal translocation to the edible parts of plants to better understand the protective mechanisms of Zn under Cd stress.

6 Data availability statement

The data that supports this research will be shared upon reasonable request to the corresponding authors.

7 Author contributions

MHR: conceptualization, investigation, data curation, and writing – original draft. MMR: funding acquisition, supervision, and writing – review & editing. MIUS: visualization, and writing – review & editing. RN: writing – review & editing. All authors approved the final version of the manuscript.

8 Conflict of interest

The authors declare no conflict of interest related to this study.

9 Ethical statements

Not applicable.

10 Copyright statement

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